

STUDIES ON PHOSPHATE UPTAKE BY MYCORRHIZAS

By

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ABSTRACT

In laboratory studies, phosphate uptake by mycorrhizas of Pinus radiata was influenced by mycorrhiza size, sheath development and physiological differences. Physiological differences were investigated by kinetic analysis methods.

Studies of phosphate uptake along roots showed that uninfected, rapidly elongating parts of long roots are as efficient as mycorrhizas over short periods. However these long root areas differ from mycorrhizas in that they are highly efficient for only a relatively short time while mycorrhizas are functional in approximately the same position in the soil for several months. From a consideration of ion movement to roots, it was advanced that under some conditions mycorrhizas can thus feed from relatively large volumes of soil without the necessity for hyphal growth into the soil. Data on distances between roots in soil showed the scope for such long range feeding. Other aspects of persistence in function are discussed.

Criteria for study of the use of insoluble inorganic phosphates and of organic phosphates by mycorrhizas were examined. Analysis of P. radiata litter showed 80 to 93 percent of the litter phosphorus to be in a number of organic forms.

Preliminary studies with vesicular-arbuscular mycorrhizas showed similar behaviour of these to ectotrophic mycorrhizas in some aspects of phosphate absorption.

INTRODUCTION

Most virgin Australian soils are phosphate deficient (Jackson 1966)

and Raupach (1967) cited several instances of response of forests to phosphate applications. The frequently encountered phosphate deficiencies in Australian forests have led to studies on the functions of mycorrhizas in phosphate uptake though this is only one of their probable many functions. This paper describes some of the physiological approaches being used, and attempts to relate laboratory studies to probable field situations. A new theory on the ecological importance of mycorrhizas is advanced.

The studies consider three types of phosphate supply, viz. readily soluble inorganic sources, poorly soluble inorganic sources such as apatite and rock phosphate, and organic sources such as those found in litter. The last becomes increasingly important as the young stand grows and needle fall and recirculation of nutrients from litter become significant. The emphasis is on qualitative and quantitative differences between various mycorrhizal associations and uninfected roots.

Most of the studies described below have been on ectotrophic mycorrhizas of *Pinus radiata* and only recently has detailed investigation of endotrophic mycorrhizas been commenced. The study of endotrophic mycorrhizas has been largely neglected, although the causal fungi of vesicular-arbuscular endotrophic mycorrhizas, *Endogone* spp., occur in most soils (Mosse and Bowen 1967) and on a wide range of plants. This subject in relation to forestry was reviewed by Bowen (1967).

UPTAKE OF READILY SOLUBLE INORGANIC PHOSPHATE FROM SOLUTION

General methods

Dihydrogen potassium phosphate with [^{32}P] added was used as the readily available and soluble form of phosphate. Solution concentrations of phosphate ranged from 1×10^{-7} M to 1×10^{-2} M but most were at 5×10^{-6} M, a concentration nearer to that expected in soil solution (Russell 1961) than that frequently employed in plant physiological studies. Absorption periods ranged from a few minutes to two hours and [^{32}P] was added at between 60 and 300 $\mu\text{Ci/l}$. Uptake was carried out in aerated solutions or in shallow solution layers and after the absorption period the material was washed

briefly in water to remove all "free space" phosphate. The absence of physical adsorption of phosphate in the systems under study was confirmed by subsequent washing with phosphate and by the use of metabolic inhibitors such as low temperatures (4°C) or 10^{-3} M potassium cyanide.

Counting of radio-activity of roots and mycorrhizas was by mica end-window Geiger-Muller tube or by gas flow chromatogram scanner (Bowen and Rovira 1967). Self absorption of the relatively energetic emissions of [^{32}P] is negligible in such systems. Absolute values of phosphate uptake were derived from counts obtained from an aliquot of the solutions.

Differences in phosphate uptake between mycorrhizal types and uninfected roots¹

Differences in tree growth with different mycorrhizal fungi have been shown (Moser, 1956, Levisohn, 1957, see also Table 5, this paper) but reasons for these have been little studied. Differences between beech mycorrhizas in short term phosphate uptake were ascribed to the extent of fungal sheath development (Harley and McCready 1950).

In our studies excised mycorrhizas and short, presumably uninfected, roots² were collected from a forest and a forest nursery and aerated in distilled water for 18 hours at 25°C prior to uptake from 1×10^{-3} M phosphate, buffered at pH 5.5, for two hours. [^{32}P] was added at 60 $\mu\text{C}/\text{l}$. Different mycorrhizal types were assumed on the basis of gross morphological differences such as colour, type of forking and size, and in order to exclude effects of environment on the morphological appearance, material was taken

¹ The data in this and the following section were presented by Bowen (1962). Copies of this paper "The Uptake of Phosphate by Mycorrhizas of Pinus radiata", Paper 97, 1-6, Third Australian Conference in Soil Science, Canberra 1962 are available from the author.

² In work described in this section, roots were presumed uninfected on the basis of absence of forking and absence of a fungus sheath. This is not an infallible guide to non-infection.

from the same area, frequently from the same root. Samples of 25 mycorrhizas (5 - 7 mg dry weight) were sufficient for reliable uptake data and uptake was carried out in triplicate where possible. Although the results in Table 1 were obtained on excised material, similar differences have been obtained using intact seedlings and shorter uptake times to reduce errors arising from the translocation of absorbed phosphate to other parts of the plant.

Examination of uptake per mycorrhiza (Table 1) shows that all types of mycorrhizas absorbed more phosphate than did uninfected short roots, in agreement with the findings of Kramer and Wilbur (1949) and of Harley and McCready (1950). There were also large differences between mycorrhizal types, Kuitpo (ii) absorbing five times as much phosphate as Kuitpo (i) which took up twice as much phosphate as did uninfected short roots collected from the same site.

TABLE 1

THE UPTAKE OF PHOSPHORUS BY EXCISED MYCORRHIZAS OF P. RADIATA

Uptake from 1×10^{-3} M dihydrogen potassium phosphate for 2 hours
at 25°C, pH buffered at 5.5.

Collection Site	Type	Phosphorus Uptake * (10^{-8} moles P.)	
		Average per root	Average per mg D.W.
Kuitpo Forest	(i) sheath well developed	0.70*	7.5
	(ii) " " "	3.5	15.5
	Uninfected short lateral root	0.4	3.5
Belair Nursery	(iii) sheath well developed	2.5	15.0
	(iv) sheath poorly developed	1.5	5.5
	Uninfected short lateral root	0.7	5.5

* Mycorrhizal uptake expressed as uptake per forked root.

Examination of efficiency of uptake, i.e. uptake per mg, showed that:

(a) in the Kuitpo material both types of mycorrhizas were more efficient than were uninfected roots but there was only a two-fold difference in efficiency between the two mycorrhiza types. The sheaths of these two types were comparable in thickness and structure, but the mean size of type (ii) was much greater than that of the other. It is concluded that in this instance differences in uptake were due to size of mycorrhiza and to 'physiological' differences;

and (b) in the Belair samples, type (iv) had only the same efficiency of uptake as the uninfected short roots. Although this mycorrhizal type had a well developed Hartig net there was almost no sheath development, and this data confirms that of Harley and McCready (1950) on the importance of fungus sheath development in uptake of phosphate. It also emphasizes that although uptake by mycorrhizas may be greater than that of uninfected short roots, in some cases this may be due only to size. It is well not to forget the importance of the increase in surface area of mycorrhizas over that of uninfected roots indicated by Hatch (1937).

A well stirred laboratory solution is a poor representation of the soil solution around a root in soil or litter and it must be emphasized that laboratory differences in phosphate uptake from solutions do not necessarily mean that differences in tree stimulation will occur in the field, although they may indicate the possibility of this. In some cases, the less efficient mycorrhizas may still be able to raise the phosphate uptake of the plant to such a level that a factor other than phosphate becomes limiting.

Kinetic analyses of phosphate uptake

To give one interpretation of 'physiological' differences in phosphate uptake between the mycorrhiza types designated as Kuitpo (i) and (ii), the kinetic analysis approach to nutrient uptake was used. This was first applied by Hagen and Hopkins (1955) to phosphate uptake by barley roots. The basis is that since phosphate uptake is metabolically mediated, phosphate may be envisaged as entering the cell in association with a carrier which subsequently dissociates from the phosphate and is available once more

for phosphate transport. A kinetic analysis analogous to the Michaelis-Menten analysis of enzyme reactions is then employed to define uptake in terms of the number of carriers, the avidity of the carrier for the phosphate and dissociation of phosphate and carrier. Although subject to objections, there is likely to be more validity in this approach for phosphate absorption which is almost entirely 'active uptake', than for cation uptake, where cation exchange, adsorption to cell walls, and the possibility of passive or of active uptake considerably complicate the interpretation of kinetics. We must emphasize that there may be other interpretations of the kinetics than that given below (Briggs, Hope and Robertson 1961).

Mycorrhizal types Kuitpo (i) and (ii) were aerated for 20 hours at 25°C in distilled water before a ten minute uptake period from concentrations of phosphate ranging from 1×10^{-7} M to 1×10^{-2} M adjusted to pH 4.0. Some of the material was given uptake periods ranging from two minutes to 15 minutes in 5×10^{-4} M and in 5×10^{-6} M phosphate.

Curves obtained with the Hofstee plot (Hofstee 1952), Fig. 1, were similar to those obtained with barley, millet and lucerne by Noggle and Fried (1960) and suggest that with both mycorrhizal types uptake of phosphorus from orthophosphate may be through at least two carrier systems. Hagen (1956) has shown that with barley roots these could correspond to uptake of $H_2PO_4^-$ and HPO_4^{2-} ions (systems "a" and "b" respectively). Calculation of the apparent dissociation constant (analogous to the Michaelis constant) indicating the affinity between phosphate (P) and the phosphate carrier (R), the rate constants for the dissociation of the phosphate-carrier complex (RP) to release phosphorus inside the plant and the concentration of phosphate carriers (ΣR) gave the data in Table 2.

The results indicate that the differences in phosphate uptake may be defined in terms of a greater affinity between both types of phosphate ions in solution and their carriers (R_a and R_b) with mycorrhizal type (ii) than with type (i) and a greater concentration of phosphate carriers with (ii). On the other hand the dissociation constant for the release of phosphate in the cell was somewhat less with (ii) than with (i). However, more study is necessary to confirm this interpretation of the kinetics of phosphate absorption.

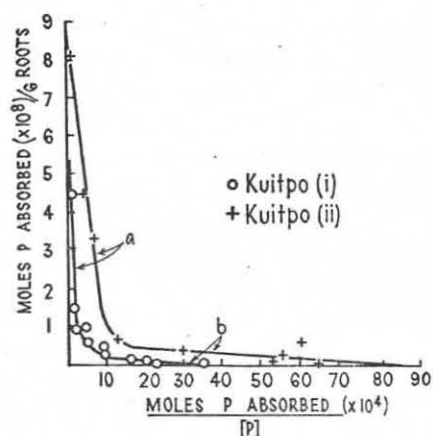


Fig. 1. Hofstee plots of phosphate uptake by two mycorrhiza types, Kuitpo (i) and Kuitpo (ii). Portions a and b are due mainly to the two carriers "a" and "b" respectively (see text).

TABLE 2

KINETIC "CONSTANTS" FOR PHOSPHATE UPTAKE BY TWO MYCORRHIZAL TYPES

Criteria	Mycorrhizal Type	
	Kuitpo (i)	Kuitpo (ii)
(i) Apparent dissociation constants		
$K_{ma} \times 10^4$	33	10.4
$K_{mb} \times 10^6$	6.0	3.7
(ii) Rate constants (Mole P $\times 10^3$ /mole RP/sec.)		
K_a	7.8	9.9
K_b	9.4	4.5
(iii) Concentration of carriers (Moles $\times 10^8$ /g)		
ΣR_a	1.2	1.4
ΣR_b	3.3	8.1

Uptake of phosphate along pine roots

Roots of *P. radiata* grown in soil in pots were used to define the regions of phosphate uptake. They were pretreated in 5×10^{-4} M calcium sulphate for two hours before uptake for 15 minutes from a solution of 5×10^{-4} M calcium sulphate containing 5×10^{-6} M phosphate and [^{32}P] at 300 $\mu\text{C}/\text{l}$. The distribution of [^{32}P] was recorded by a method developed by Bowen and Rovira (1967) in which the roots were laid on a continuous strip of 4 cm wide chromatogram paper, covered with cellulose tape and the distribution of radioactivity recorded by means of a gas flow, chromatogram scanner. In short uptake periods the position of phosphate accumulation was essentially the position of uptake as little redistribution due to translocation occurred.

Figure 2 shows the pattern of phosphate uptake along a root bearing mycorrhizas and one having only uninfected short lateral roots. Uptake is directly proportional to the area under the corresponding part of the scan. A high uptake occurred in the regions of the mycorrhizas, as found by Kramer and Wilbur (1949) using autoradiographic methods with *P. taeda* and *P. resinosa* and uptake periods of 3 to 4 hours. Minor peaks in uptake frequently occurred with uninfected short lateral roots, a point also observed by Kramer and Wilbur. They also noted a peak in uptake behind the apex of the long root, as did Lundeberg (1961) using *P. sylvestris* and uptake periods of 6 hours. This is confirmed in Fig. 2 with *P. radiata*, quite uncomplicated by subsequent transport of isotope from the region of uptake which may occur with long absorption periods. The data in Fig. 2a (and that from other experiments not reported here) calculated as uptake per mg tissue indicated that the efficiency of uptake in the apical, uninfected, rapidly elongating area of the root was of the same order as that of mycorrhizas, in these short term studies.

MYCORRHIZAL UPTAKE OF READILY SOLUBLE PHOSPHATE FROM SOIL

It was shown above that the rapidly elongating uninfected regions of *P. radiata* roots are as efficient in phosphate uptake as are mycorrhizas,

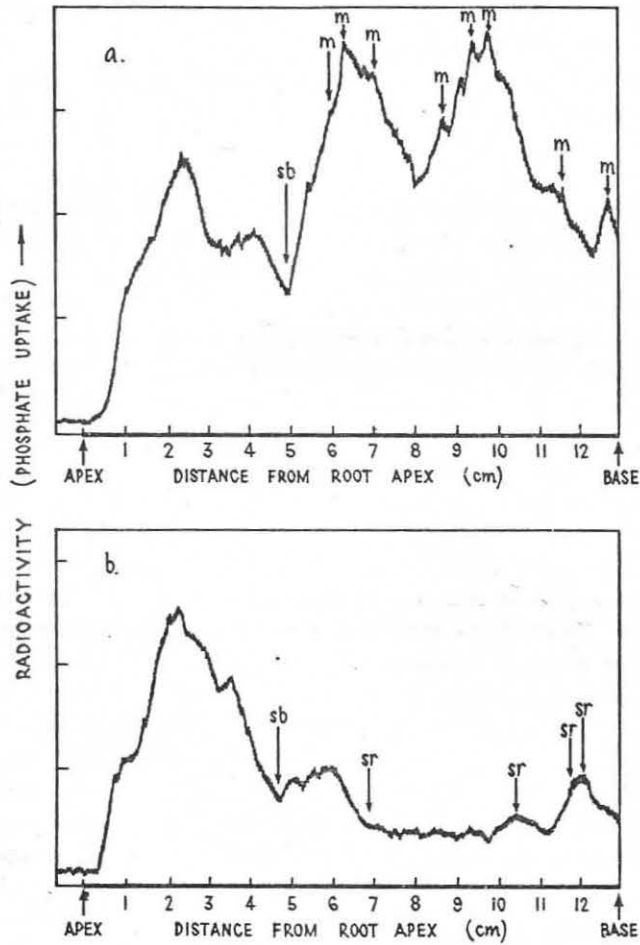


Fig. 2. Uptake of phosphate along (a) *P. radiata* roots bearing mycorrhizas and (b) uninfected *P. radiata* roots, over 15 minutes from 5×10^{-6} M phosphate containing $[^{32}\text{P}]$. m: positions of mycorrhizas, sr: position of uninfected short roots, sb: commencement of suberization.

at least over short uptake periods (it is not yet known for how long this holds). Beech mycorrhizas can maintain the initial high uptake rates for some hours (Harley et al. 1956) and this forms the basis of the hypothesis advanced by Harley (1959) that in soil, mycorrhizas may function as efficient uptake organs in competition with soil micro-organisms and store nutrients (especially phosphate) following any sudden release of these. Such trapped nutrients are subsequently passed to the tree. Sudden release of readily available nutrients occurs in soils with accession of litter, leaf leachates (Will 1959), and fluctuating conditions such as wetting and drying (Birch 1958).

The existence of zones of high uptake in uninfected elongating roots led Bowen (1967) to point out that the implications of one of the major differences between such parts of uninfected roots and mycorrhizas has been overlooked. This difference is, that while the highly absorbing part of an uninfected elongating root is of relatively short duration in a particular position (probably only some seven days - Bowen, unpublished data), mycorrhizas can remain active in approximately the same position in the soil for long periods. Harley (1959) cited the longevity of a mycorrhiza system in pine of from several months to a year. The implications of longevity in function can be best understood if one considers the ways in which solutes move to roots. A consideration of this also helps to relate laboratory uptake studies from solutions, to the field situation.

The limiting factor in ion uptake from soil is frequently not the ability of the root to absorb nutrients but the rate of movement of the ion to the root, a point which can be readily checked by comparison of ion uptake rates from well stirred solutions and from soil. Solutes move to roots by means of diffusion along a concentration gradient (or an electrochemical potential gradient) or by bulk flow in water (referred to as convection). Diffusion is the most important method for phosphate movement (Olsen, Kemper and Jackson 1962). Factors such as the concentration gradient between soil solution and root, chemical reactions with soil and the tortuous path through soil will affect diffusion rates. The distance over which

phosphate diffusion is important is further affected by the rate of replenishment of solution phosphate from that in the solid phase in a soil.

The distance over which solutes will diffuse to a root in soil is approximately proportional to the square root of the time the root is functional in uptake of that solute but this can be modified greatly by the factors mentioned above and by interaction between diffusion and convection (Gardner 1965). An organ functioning for a long period, such as a mycorrhiza, can thus feed from a much larger volume of soil than one with a short functional life, such as the elongating portion of an uninfected root. Olsen, Kemper and Jackson (1962) calculated that in a silty clay loam, phosphate in soil one mm from a maize root would contribute to its nutrition over 10 days, but that this would be five mm had the root been functional in that position for 100 days. This leads to a very great increase in the volume of soil which could contribute phosphate to the root system, regardless of whether one considers movement to a cylinder or to a point (or small sphere). The latter is probably a closer approximation to a pine mycorrhiza in soil. It should be remembered that not all of the increased soil volume will contribute phosphate equally to the root, as phosphate contributed decreases with distance.

In a soil low in phosphate and in replenishing power from the solid phase, longevity in function will play a large part in allowing diffusion over a wide radius from the mycorrhiza. The high potential rate of uptake by mycorrhizas (as shown by laboratory experiments in solutions) will play a role by helping determine the concentration of phosphate in soil solution at the mycorrhiza surface, and therefore the gradient which is the 'driving force' for diffusion. In a soil high in phosphate and with rapid replenishment of this phosphate from solid phase reserves, radius of diffusion is likely to be small but the persistence of mycorrhizas and their maintenance of a high uptake rate will tend to lead to low solution concentrations of phosphate, encouraging release of phosphate from the solid phase. It is the former case which is probably more relevant to the low fertility soils in which tree response to mycorrhizal infection is most likely to occur. Although phosphate has been considered particularly above, the

same general principles can be applied to all solutes moving by diffusion e.g. potassium (Barber et al 1962).

Where solutes move mainly in bulk flow of water, such as nitrate and sulphate (Moodie 1965) the implications of longevity of mycorrhizas is much the same as for diffusion, but there is the added requirement that mycorrhizas be water uptake organs as well. The distance solutes move by convection is approximately proportional to the square root of time in unsaturated soils, the usual situation (Todd 1959) and proportional to time in saturated soils (Gardner 1965). Ions can be moved large distances by convection. There has been little study of the water uptake functions of mycorrhizas, but there is no obvious reason why they should not act in this way and Cromer (1937) observed a faster recovery by mycorrhizas of *P. radiata* than by uninfected roots on the addition of water to a dry soil.

It is apparent that longevity in function of mycorrhizas involves ecological advantages and will often mean feeding from a considerable volume of soil, without the necessity of hyphal growth into the soil. In some situations, hyphae enable feeding direct from a large volume of soil as suggested by Björkman (1949) and Melin and associates (e.g. Melin and Nilsson 1950) and where extensive hyphal development into soils occurs from mycorrhizas this may supplement the consequences of longevity in function outlined above. Although hyphal growth from mycorrhizas into soil is observable in some instances, this is not always so and it probably differs with the particular soil conditions and the fungus; important questions are: How common is extensive hyphal growth from mycorrhizas and under what conditions and to what distance does it occur? There are surprisingly little data on this. In some mycorrhizas examined in this laboratory mycelia moved little more than 0.1 mm from the mycorrhizal sheath; nevertheless even this small distance can be important in nutrient uptake from soil.

Both the 'longevity' and 'hyphal exploration' hypotheses of mycorrhiza function in soil must be considered in relation to the root distribution of the tree. Again, there is little published quantitative data on this. Table 3 indicates some results of current studies on distances between

P. radiata roots in soils and distances between short roots and/or mycorrhizas along a root in the surface 12 cm of soil. This was the position of maximum root concentration of P. radiata in the stands examined by Bowen (1964). It is seen that appreciable distances are involved and that any way in which the root can feed beyond its immediate micro-environment in a deficient soil would be of importance.

TABLE 3
ROOT DISTRIBUTION OF P. RADIATA

Sample details	Long Roots			Short Lateral Roots		
	Mean ¹ distance apart ² (mm)	Closest ³ Range (mm)	root Mean (mm)	Closest short root ⁴ Range (mm)	Mean (mm)	Length (mm)
Lateritic podzol ⁵	12.8	2-25	7.6	1-52	9.0	4.4
sandy solonized solonetz ⁶	14.2	2-31	9.0	1-44	5.9	4.5

1. Means of at least 220 roots.
2. Based on number of long roots in 15 x 15 x 12 cm samples.
3. Distance to the nearest neighbouring long root.
4. Including uninfected short roots and mycorrhizas, and measured along individual long roots.
5. 0-12 cm under 27 yr. P. radiata.
6. 0-12 cm under 20 yr. P. radiata.

Over a period of, say, 100 days, more phosphate would diffuse to the highly efficient zone of elongating roots as it 'moves' through the soil than to a stationary mycorrhiza. Although this is important, efficient use of the soil by growing roots necessitates small distances between roots, which does not seem to be the usual case with P. radiata (Table 3) although it may occur with grasses. With this tree species, efficient use of soil is aided by mycorrhizal longevity and by hyphal exploration, where it occurs.

UPTAKE OF POORLY SOLUBLE INORGANIC PHOSPHATE

Although Jahn (1934), Routien and Dawson (1943) and Schatz et al. (1954) proposed that excretion of acid, hydrogen ions, or chelating compounds respectively by mycorrhizas could have a solubilizing effect on poorly soluble phosphates these ideas have not been experimentally tested.

TABLE 4

SOLUBILIZATION OF ROCK PHOSPHATE BY MYCORRHIZAL
FUNGI IN PURE CULTURE

Fungus	P released mg.*	Final pH of medium*
<u>Boletus granulatus</u>	2.28	4.6
<u>Cenococcum graniforme</u>	1.94	4.8
<u>Rhizopogon luteolus</u>	1.85	4.4
<u>Boletus luteus</u>	1.24	6.0
Uninoculated		
pH 4.0	1.00	4.4
pH 6.5 (initial)	0.14	6.5

* Means of 4 replicates.

Table 4 demonstrates the release of phosphate from rock phosphate in three weeks by three mycorrhizal fungi inoculated into Melin-Norkrans solution (Melin 1959) with rock phosphate replacing the potassium dihydrogen phosphate. Although there was no direct correlation between acid production (which occurred with all fungi) and phosphate released, Johnston (1952) and Sperber (1958) have shown that acids vary in their ability to solubilize such inorganic forms of phosphate as tricalcium phosphate and hydroxyapatite. In such laboratory experiments where a good supply of carbohydrates are available (see also Rosendahl 1943) it is probable that solubilization is due solely to acid production and this may not occur under field conditions. It is unwise to extrapolate such laboratory experience to a field situation.

Stone (1950) attempted to show the solubilization of mineral phosphates by mycorrhizas by growing Sudan grass in association with mycorrhizal P. radiata in pots. The grass did not show an increase in phosphate uptake, but this cannot be taken as proof of non-solubilization as any solubilized phosphate may have been rapidly absorbed by the mycorrhizas. Table 5 summarizes an experiment in which rock phosphate was incorporated into a phosphate deficient sand in pots and inoculated and uninoculated P. radiata grown in them under glasshouse conditions for 14 months. The sand was heat sterilized before planting, but was one in which little phytotoxicity developed as a result of heating. No attempt was made to keep the pots sterile.

Table 5 shows that both mycorrhizal and non-mycorrhizal plants responded to addition of rock phosphate, a finding also made by Stone (1950) with four mineral phosphates. This response could be due to solubilization by rhizosphere micro-organisms in association with non-mycorrhizal roots or with the fungal sheath of mycorrhizas (the 'hyphosphere') and it is clearly necessary that all studies of the use of poorly available substrates by mycorrhizas themselves, should employ plants grown aseptically except for the mycorrhizal fungus, if unequivocal results are to be obtained. Table 5 also shows different plant stimulation by different mycorrhizal fungi; no response was obtained after inoculation with Boletus luteus, the species used by Stone (1950) with P. radiata with no success. Plant

growth in uninoculated, but fertilized pots, was superior to that in mycorrhizal unfertilized pots in this deficient soil. The addition of rock phosphate increased mycorrhiza formation in inoculated pots.

TABLE 5

RESPONSE OF INOCULATED AND UNINOCULATED PINUS RADIATA SEEDLINGS TO
ROCK PHOSPHATE

Seedlings were grown in Mt. Burr Sand (A₂ horizon) for 14 months.

Rock phosphate was added at the rate of 0.56 g/3.5 kg air dry
soil (approximately equivalent to 376 kg/ha).

Inoculum	Dry Weight/ seedling*		P content/ seedling*		Mycorrhiza %	
	No P	g. Rock phosphate	No P	mg. Rock phosphate	No P	Rock phosphate
<u>Boletus</u>						
<u>granulatus</u>	2.15	4.55	0.75	2.61	12	33
<u>Rhizopogon</u>						
<u>luteolus</u>	2.65	4.42	1.10	1.77	19.5	40.5
<u>Boletus luteus</u>	1.45	3.60	0.49	1.79	16	42.5
<u>Cenococcum</u>						
<u>graniforme</u>	1.58	3.70	0.58	1.42	2	20.5
Uninoculated	1.65	3.65	0.58	1.50	6	4
	LSD=0.57, P=0.05		LSD=0.69, P=0.05		LSD=20, P=0.05	
	LSD=0.77, P=0.01		LSD=0.92, P=0.01		LSD=27, P=0.01	

* Means of four replicate pots with 5 seedlings each.

UPTAKE OF ORGANIC PHOSPHATES

The use of a number of organic compounds by pure cultures of mycorrhizal fungi has been demonstrated by various workers (Harley 1959, Melin 1954, Melin and Das 1954). Although Frank (1894) suggested the mycorrhizal use of organic nitrogenous compounds in litter, there has been little critical study of this, apart from that of Hatch (1937) who found that uninfected P. strobilus could use nucleic acid and peptone as sole nitrogen sources.

Theodorou (unpublished data) has demonstrated the ability of cultures of Rhizopogon luteolus, Boletus granulatus, B. luteus, and Cenococcum graniforme to obtain phosphate from calcium and sodium phytates, but not from ferric phytate. Acidity did not solubilize the calcium and sodium phytate and it is likely that the fungi produced a phytase. It is reasonable perhaps to extrapolate laboratory findings on the use of organic substrates by mycorrhizal fungi to possible field behaviour. However, there is no real substitute for critical testing using mycorrhizas produced under microbiologically controlled conditions and with uninfected sterile plants as controls.

It is pertinent to ask whether use of particular organic substrates by mycorrhizal fungi has any relevance to the field situation. The presence of phytates in P. radiata needles and litter has been shown by electrophoresis, in this laboratory. Fractionation of litter from a 26 year stand of P. radiata, using the methods of Martin and Morton (1956) has given the data in Table 6. Eighty to 93 percent of the phosphate was in organic forms, depending on the stage of decomposition. The total phosphorus content of litter decreased by 53% with decomposition. The proportion of the protein phosphorus fraction increased with decomposition, the acid labile and nucleic acid fractions decreased whilst the acid stable and lipid fractions remained approximately the same.

Obviously once appreciable litter fall has commenced, the rate of use of organic phosphate and a mycorrhizal short-circuiting of 'classical' mineralization pathways could be important in a phosphate deficient soil. The use of the various classes of phosphate compounds indicated in Table

6, as energy sources and phosphate sources for mycorrhizal fungi in pure culture and in association with P. radiata is at present being studied further.

TABLE 6

PHOSPHATE FRACTIONATION OF PINUS RADIATA LITTER

Fraction	Green Needles	Freshly Fallen (L)	Decomposed (F)	Decomposed (H)
(a) <u>Organic and inorganic phosphate in litter</u>				
Total P mg/g D.W.	1.03	0.91	0.70	0.48
Inorganic %	20	12	12	7
Organic %	80	88	88	93
(b) <u>Distribution of organic phosphorus compounds as percentages of total phosphorus</u>				
Acid labile	18	18	8	7
Acid stable	10	8	6	6
Lipid	12	14	14	16
Nucleic acid	13	24	18	14
Protein	23	19	36	45
Residue	4	4	7	5

ENDOTROPHIC MYCORRHIZAS

Very few experiments on the uptake of soluble sources of phosphate have been carried out with endotrophic mycorrhizas. Data of Bowen and Mosse (in preparation) such as that given in Table 7 demonstrates a greater uptake of orthophosphate by parts of subterranean clover and onion infected with vesicular-arbuscular mycorrhizas. This confirms similar data by Gray

and Gerdemann (1967) on tulip-tree seedlings. Bowen and Mosse also carried out auto-radiography of thin sections which indicated a much higher incorporation of phosphate into fungus structures than into neighbouring cells when fed with 5×10^{-6} M phosphate in calcium sulphate for two to four hours. Much of the increased uptake of phosphate was due to that by the fungus and not to the fungus stimulating uptake by the root cells. Hyphae and germinating spores of *Endogone* were also shown to absorb phosphate. The fundamental questions of phosphate uptake by vesicular-arbuscular mycorrhizas would appear to be common with those for ectotrophic mycorrhizas namely phosphate uptake, storage, translocation, and release to the higher plant.

TABLE 7

UPTAKE OF PHOSPHATE BY ENDOTROPHIC MYCORRHIZAS OF

SUBTERRANEAN CLOVER

Uptake from 5×10^{-6} M Calcium Sulphate + 1×10^{-5}

M Potassium Dihydrogen Phosphate + [^{32}P] for 30 minutes.

	Phosphate uptake/mg D.W. Root. (Counts/Sec.)
Uninoculated	177
Inoculated	436***

*** Difference significant at 0.1% level.

As endotrophic fungi are functional well behind the actively growing and elongating portion of the root, the considerations of longevity, high efficiency of uptake, and the exploration of soil by the hyphae (Mosse 1963, stated that mycelium may extend up to 1 centimetre from the root) are pertinent to this type of association as well as to ectotrophic mycorrhizas.

CONCLUSIONS

1. In short term studies, mycorrhizas of *P. radiata* differ in phosphate uptake. These differences which were up to five fold, were due to physical factors, such as size, and extent of development of fungal sheath and to physiological factors. Some mycorrhizas may be no more efficient in phosphate uptake than uninfected short lateral roots.

2. Michaelis-Menten kinetic analyses can describe physiological differences between mycorrhizas as due to differences in numbers of phosphate carriers and to differences in affinity between phosphate and carriers. The data suggests a possibility of at least two phosphate carriers or transport systems in mycorrhizas, as has been suggested for roots of agronomic plants.

3. With short uptake times the rapidly elongating portion of uninfected long roots of *P. radiata* have approximately the same efficiency of phosphate uptake as do mycorrhizas.

4. Whereas efficient portions of uninfected roots are functional in phosphate uptake for only a matter of days, mycorrhizas may be functional in uptake for several months. A consideration of solute movement to roots suggests that because of their longevity in function, even in the absence of hyphal outgrowth into soil mycorrhizas may feed from much larger volumes of soil than the efficient uninfected parts of roots which remain highly absorptive for much shorter periods. Where extensive hyphal exploration of soil occurs it may supplement the importance of longevity. More quantitative information is needed on hyphal exploration of soil.

Quantitative data on root distribution indicates the ecological importance of long range feeding because of longevity of mycorrhizas and hyphal exploration of soil.

5. No conclusive proof of the use of poorly-soluble, inorganic phosphates or of organic phosphates by axenic mycorrhizas has yet been presented. Extrapolation from laboratory experiments with fungal cultures is unwise and use of synthesized mycorrhizas with all other micro-organisms absent is necessary for unequivocal results. Most of the phosphate in recently

fallen *P. radiata* needles and decomposing litter is in various organic forms so that the question of use of organic phosphates by mycorrhizas is important.

6. Experiments showing increased phosphate uptake by endotrophic mycorrhizas suggest that the basic questions of phosphate uptake, storage, translocation and transfer to the higher plant are important to this association as well as to ectotrophic mycorrhizas.

A number of aspects of phosphate relations of mycorrhizas have been considered above. Although phosphate has been studied by a number of mycorrhiza workers there is much still to be elucidated. It must be remembered however that to regard mycorrhizas only as phosphate uptake organs is as unrealistic as regarding any type of root as important only in phosphate uptake.

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